

Research Article

Assessment of Oral Biological Markers in Root Carious Lesion and Periodontitis Manifestation Traits: An In-Vitro Cross Sectional Analytical Study

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ABSTRACT

Background: Root carious lesion and Periodontitis frequently coexist because of shared clinical and biological factors, including gingival recession, exposed root surfaces, microbial dysbiosis, inflammation, altered salivary characteristics, and impaired antioxidant defenses. Oral biological markers may provide additional information regarding disease activity and the relationship between these two conditions.

Objective: To assess oral biological markers in root carious lesions and periodontal manifestation traits and to determine their association with root caries, periodontal disease, and the coexistence of both conditions.

Methods: This An In- Vitro Cross Sectional Analytical Study was conducted in the Department of Operative Dentistry and Endodontics, Shahida Islam Medical Complex, Lodhran, from 1st January 2025 to 30th June 2026 for a period of 18 months (One and a half year). A total of 430 participants were included. Root carious lesions characteristics and periodontal parameters, including plaque index, gingival index, bleeding on probing, probing pocket depth, clinical attachment loss, and gingival recession, were recorded. Unstimulated whole saliva was analyzed for pH, flow rate, vitamin C, total antioxidant capacity, IL-1 β , IL-6, TNF- α , and MMP-8. Data were analyzed using IBM SPSS Statistics version 32.0. Appropriate comparative tests, correlation analysis, and multivariable logistic regression were applied, with $p < 0.05$ considered statistically significant.

Results: Root carious lesion was identified in 186 (43.3%) participants, while Periodontitis was present in 258 (60.0%). Both conditions coexisted in 139 (32.3%) participants. Individuals with root carious lesion had significantly greater probing pocket depth, clinical attachment loss, gingival recession, plaque index, and bleeding on probing than those without root carious lesion (all $p < 0.001$). Salivary pH, salivary flow rate, vitamin C, and total antioxidant capacity were significantly lower in participants with root caries, whereas IL-1 β , IL-6, TNF- α , and MMP-8 were significantly elevated. The highest MMP-8 concentration was observed among participants with both root carious lesion and periodontitis. Multivariable analysis showed that periodontitis, gingival recession, lower salivary pH, and elevated MMP-8 were independently associated with root carious lesion.

Conclusion: Root carious lesion was significantly associated with adverse periodontal characteristics and alterations in the oral biological environment. Coexisting root carious lesion and periodontitis demonstrated the most pronounced inflammatory and antioxidant imbalance. Salivary biomarkers, particularly MMP-8, together with conventional clinical parameters, may have potential value in integrated risk assessment, although longitudinal validation is required before routine clinical application.

Keywords: Root Carious Lesion (RCL), Periodontitis, Salivary Biomarkers, MMP-8, IL-18, Oxidative Stress, Gingival Recession.

Clinical Relevance

Root carious lesion (RCL) and periodontitis frequently occur in an oral environment characterized by exposed root surfaces, microbial dysbiosis, inflammation, alteration of salivary protective mechanisms, and progressive destruction of dental or periodontal tissues. Conventional clinical examination identifies established disease but may provide limited information regarding the biological activity underlying lesion development and periodontal destruction. Assessment of salivary inflammatory and antioxidant biomarkers may therefore provide additional information about disease activity and the biological relationship between root caries and periodontal manifestations. The present research study is highly clinically relevant as it evaluates a combination of salivary characteristics, inflammatory mediators, and antioxidant markers rather than relying on a single biological indicator. Identification of a distinct biomarker profile among patients with simultaneous root caries and periodontitis may contribute to earlier risk recognition and support more individualized preventive and minimally invasive approaches to oral healthcare.

INTRODUCTION

Dental caries remains a highly prevalent multifactorial oral disease resulting from interactions between the dental biofilm, dietary substrates, host characteristics, saliva, and time. Root caries represents a clinically important form of the disease in which demineralization develops on exposed cementum or dentin, particularly in individuals with gingival recession and periodontal attachment loss. Retention of natural teeth into later life has increased the number of exposed root surfaces at risk, making root caries an increasingly important concern in adult and older populations (1,2). Saliva has an essential protective role through clearance, buffering, remineralization, antimicrobial activity, and maintenance of oral homeostasis. Alterations in its physicochemical and

molecular composition may therefore contribute to the development or progression of carious lesions. Recent systematic reviews have suggested that salivary components have potential value as complementary biomarkers for identifying dental caries, although heterogeneity between studies continues to limit the establishment of universally accepted diagnostic thresholds (3,4).

Management of root caries has progressively shifted toward prevention, lesion arrest, tissue preservation, and minimally invasive treatment. Fluoride-based strategies remain important because exposed dentin and cementum are particularly susceptible to demineralization. A systematic review by Mungur et al. demonstrated the potential of silver diamine fluoride for the management of carious lesions in permanent teeth, including root lesions (5). More recently, Pervez et al. evaluated silver diamine fluoride as a conservative approach to root caries and reported greater caries-arrest effectiveness with 38% silver diamine fluoride than with sodium fluoride varnish, further supporting minimally invasive approaches for preservation of affected root surfaces (6). The increasing emphasis on biologically conservative dentistry is also consistent with the broader concept of maintaining peri-alveolar tissue integrity and minimizing unnecessary tissue trauma (7).

Periodontitis is a chronic inflammatory disorder characterized by dysbiotic microbial challenge, an altered host response, progressive clinical attachment loss, periodontal pocket formation, and destruction of supporting alveolar tissues. Gingival recession and loss of attachment associated with periodontal disease can expose the root surface, creating an anatomical environment susceptible to root caries. At the same time, the inflammatory response associated with periodontitis produces measurable changes in saliva. Consequently, saliva is increasingly being investigated as a non-invasive diagnostic fluid capable of reflecting local inflammatory and tissue-destructive activity. Reddahi et al. reported higher salivary concentrations of IL-

1 β , IL-6, and MMP-8 among patients with periodontitis, while a study conducted in a Pakistani population also demonstrated increased salivary MMP-8 and IL-1 β in periodontal disease (8,9). Broader systematic evidence similarly supports the diagnostic potential of protein-based salivary biomarkers for distinguishing periodontal health from disease (10).

Among the biomarkers investigated for periodontal disease, matrix metalloproteinase-8 (MMP-8) has received particular attention because of its role in extracellular matrix and collagen degradation. A recent systematic review and meta-analysis demonstrated substantially higher salivary MMP-8 concentrations in individuals with periodontitis than in periodontally healthy controls and also found associations with clinical periodontal parameters (11). Other inflammatory mediators, including IL-1 β , IL-6, and TNF- α , participate in host inflammatory pathways involved in periodontal tissue destruction and may complement MMP-8 when biological disease activity is being evaluated. Contemporary biomarker research therefore increasingly favors multimarker profiles over reliance on a single molecular indicator. Advances in salivary proteomics further support the possibility of identifying disease-specific molecular patterns that may eventually complement conventional periodontal examination (12,13).

Oxidative stress represents another important biological mechanism linking host inflammatory activity with oral tissue damage. An imbalance between reactive oxygen species and antioxidant defense can contribute to periodontal tissue injury, while total antioxidant capacity provides an integrated estimate of the ability of biological fluids to counter oxidative challenge. A recent meta-analysis found greater oxidative stress and generally reduced antioxidant capacity in patients with periodontitis compared with healthy individuals, supporting the importance of redox imbalance in periodontal pathogenesis (14). Vitamin C is an important water-soluble antioxidant involved in collagen synthesis, tissue repair, and maintenance of periodontal integrity. Mahajani et al. investigated vitamin C in patients with chronic periodontitis and examined its relationship with salivary total antioxidant capacity, providing evidence that antioxidant status may have clinical relevance in periodontal disease (15). More recent systematic evidence has

continued to examine ascorbic acid as a potential adjunctive factor in maintaining oral and periodontal health (16).

Although root caries and periodontal disease frequently coexist through common clinical features such as gingival recession, root exposure, plaque accumulation, altered saliva, inflammation, and changes in the local biological environment, relatively limited evidence has assessed root caries and periodontal manifestation traits simultaneously using a comprehensive panel of oral biological markers. Most available research has investigated either caries-related salivary parameters or periodontal inflammatory biomarkers separately. Furthermore, recent precision-dentistry literature emphasizes the need for standardized multimarker approaches capable of linking salivary biology with clearly defined clinical outcomes (17). Therefore, the present study was conducted to assess oral biological markers in patients with root carious lesions and periodontal manifestation traits and to determine their relationship with root-caries status, lesion burden, periodontal pocket depth, clinical attachment loss, gingival recession, and the coexistence of root caries and periodontitis. The study additionally evaluated whether simultaneous root caries and periodontal disease were characterized by increased inflammatory-marker concentrations, altered salivary characteristics, and reduced antioxidant status.

METHODOLOGY

This An In- Vitro Cross Sectional Analytical Study was conducted in the Department of Operative Dentistry and Endodontics, Shahida Islam Medical Complex, Lodhran, over a period of 18 months (One and a half year), from 1st January 2025 to 30th June 2026. The research study was designed to assess oral biological markers in relation to root carious lesions and periodontal manifestation traits and to determine whether variations in inflammatory, antioxidant, and salivary parameters were associated with the presence and severity of these oral conditions. Participants presenting to the department during the study period were screened for eligibility. Those fulfilling the predefined selection criteria were enrolled through a non-probability consecutive sampling technique until the required sample size was achieved.

Prior to commencement of the study, approval was obtained from the relevant Institutional Research and Ethical Review Committee of

Shahida Islam Medical Complex, Lodhran. The purpose, procedures, possible benefits, and requirements of participation were explained to all participants before enrollment. Written informed consent was obtained before clinical examination and collection of biological samples. Participation was voluntary, and confidentiality of all clinical and laboratory information was maintained throughout the study. Data were used exclusively for scientific and research purposes.

The sample size was 430 participants. The initial minimum sample size was calculated using the formula for estimation of a single population proportion: $n = Z^2 \times p \times (1 - p) / d^2$ where n represented the required sample size, Z was the standard normal value of 1.96 at a 95% confidence level, p represented the anticipated proportion of the outcome of interest, and d represented the desired margin of error. In the absence of a sufficiently precise local estimate for the combined biomarker profile of root caries and periodontitis, a prevalence of 50% was used to provide the maximum required sample size, with a 5% margin of error. Thus: $n = (1.96)^2 \times 0.50 \times 0.50 / (0.05)^2 = 384.16$

The minimum calculated sample size was therefore approximately 384 participants. To compensate for potentially inadequate biological specimens, incomplete clinical measurements, laboratory processing errors, and missing observations, the sample was increased by approximately 12%, giving a final target sample of 430 participants. All 430 participants included in the final analysis had sufficient clinical and laboratory information for assessment of the principal study variables. Adult patients attending the study center and having clinically assessable permanent teeth and exposed root surfaces were considered eligible for inclusion. Participants were included if they were willing to undergo oral and periodontal examination, provide an oral biological sample, and give informed consent. Patients with acute odontogenic infections requiring emergency treatment, severe systemic illness interfering with examination or specimen collection, or inadequate biological specimens were excluded. Participants who had received systemic antibiotics, professional periodontal therapy, or other treatment likely to substantially alter the oral inflammatory or microbial environment shortly before enrollment were also excluded where relevant. Samples contaminated during collection, improperly stored specimens, and those with

insufficient volume for biomarker assessment were excluded from laboratory analysis.

A structured data collection form was used to document age, sex, smoking status, diabetes mellitus, oral-hygiene practices, frequency of tooth brushing, relevant medical history, previous periodontal treatment, and previous restorative treatment for root caries. All participants underwent a standardized oral examination under adequate illumination using routine dental diagnostic instruments. Root surfaces were systematically examined for the presence or absence of root carious lesions. For each identified lesion, the affected tooth, anatomical surface, location, number of lesions, lesion activity, and clinical severity were documented. Root carious lesions were categorized as active or inactive according to their clinical appearance and surface characteristics. The distribution of lesions between anterior and posterior teeth and between the maxillary and mandibular arches was also recorded. Gingival recession and root-surface exposure were carefully assessed because exposure of the root surface represents an important clinical prerequisite for the development of root carious lesion.

Where appropriate, the Root Caries Index (RCI) was used to quantify the burden of root-surface disease. The index incorporated the number of decayed and restored root surfaces relative to the number of exposed root surfaces. Participants were additionally classified according to whether they had a single root carious lesion or multiple root carious lesions, and lesions were categorized clinically as mild/shallow, moderate, or deep according to their extent. Standardized examination procedures were maintained throughout the study to reduce variation in lesion classification. Where uncertainty existed regarding lesion activity, the clinical appearance and surface texture were considered together before final categorization.

A comprehensive periodontal examination was performed to assess the manifestation traits of periodontal disease. The periodontal variables included the Plaque Index, Gingival Index, bleeding on probing (BOP), probing pocket depth (PPD), clinical attachment loss (CAL), and gingival recession. Periodontal pocket depth was measured in millimeters from the free gingival margin to the base of the periodontal sulcus or pocket, whereas clinical attachment loss was measured in relation to the cemento-enamel junction. Gingival

recession was recorded as the distance between the cemento-enamel junction and gingival margin. Bleeding on probing was assessed following gentle periodontal probing and recorded according to the proportion of examined sites showing bleeding. The presence and clinical severity of periodontitis were subsequently categorized using the periodontal findings obtained during examination.

For comparative analysis, study participants were classified into four clinically relevant groups: participants with neither root caries nor periodontitis, participants with root caries only, participants with periodontitis only, and participants with both root caries and periodontitis. This classification allowed evaluation of whether simultaneous expression of the two diseases was accompanied by a more pronounced alteration in oral biological-marker concentrations than either condition alone.

For assessment of oral biological markers, unstimulated whole saliva was collected under standardized conditions. Participants were instructed to avoid eating, drinking except water, smoking, chewing gum, and routine oral-hygiene procedures for an appropriate period before sample collection. Saliva was collected into sterile, properly labeled containers while minimizing contamination with blood or other material. The amount of saliva collected over the specified collection period was used to calculate the salivary flow rate in mL/min. Salivary pH was measured soon after sample collection using an appropriate calibrated pH-measurement method. The samples were subsequently processed according to the requirements of the respective laboratory assays.

The biological-marker panel included parameters representing both inflammatory and antioxidant pathways. Vitamin C concentration and total antioxidant capacity were assessed to characterize antioxidant status, while inflammatory activity was evaluated using interleukin-1 beta (IL-1 β), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF- α), and matrix metalloproteinase-8 (MMP-8). The selected biomarkers were chosen because they represent clinically relevant processes involved in periodontal tissue inflammation, extracellular matrix degradation, host inflammatory response, oxidative balance, and the oral environment associated with hard-tissue disease. The biomarkers were measured using validated

biochemical or immunoassay techniques according to the manufacturer's instructions for the respective assay kits. Concentrations were recorded in the appropriate units for subsequent statistical analysis.

Quality-control measures were applied during specimen collection, storage, processing, and laboratory testing. All specimens were assigned a unique study identification code to maintain participant confidentiality and prevent identification during laboratory assessment. Sample collection procedures were standardized, and specimens showing visible contamination, insufficient volume, or processing errors were not accepted for final analysis. Laboratory procedures were performed according to standardized operating protocols, and equipment was appropriately calibrated where required. Data collection forms were reviewed for completeness before data entry, and laboratory findings were matched with clinical data using study identification numbers rather than participant names.

The primary outcome of the study was the association of oral biological-marker levels with the presence of root carious lesions. Secondary outcomes included the relationship of these markers with lesion activity and severity, number of root carious lesions, periodontal pocket depth, clinical attachment loss, gingival recession, bleeding on probing, and severity of periodontal manifestations. Particular emphasis was placed on determining whether individuals with both root caries and periodontitis demonstrated a characteristic biological profile consisting of higher inflammatory-marker concentrations, lower antioxidant capacity, and less favorable salivary conditions compared with participants having only one condition or neither condition. Data were entered and analyzed using IBM SPSS Statistics version 32.0. IBM SPSS Statistics version 32 became generally available in April 2026. Data were initially checked for completeness, coding errors, and outlying values. Continuous variables such as age, salivary pH, salivary flow rate, periodontal pocket depth, clinical attachment loss, gingival recession, vitamin C, total antioxidant capacity, IL-1 β , IL-6, TNF- α , and MMP-8 were summarized as mean $\pm$ standard deviation when normally distributed. Variables with skewed distributions were reported as median and interquartile range. Categorical variables, including sex, smoking status, diabetes mellitus, presence of root caries, lesion

activity, periodontal status, and disease categories, were presented as frequencies and percentages.

The independent-samples t-test was used to compare normally distributed continuous variables between two independent groups, whereas the Mann–Whitney U test was used when normality assumptions were not met. Comparisons involving more than two independent groups were performed using one-way analysis of variance (ANOVA) for normally distributed data or the Kruskal–Wallis test for non-normally distributed variables. Categorical variables were analyzed using the chi-square test, while Fisher's exact test was used when expected cell frequencies were small. Correlations between the number or severity of root carious lesions and continuous periodontal or biological-marker variables were assessed using Pearson's correlation coefficient for normally distributed variables and Spearman's rank correlation coefficient for non-parametric data.

A multivariable logistic regression analysis was performed to identify factors independently associated with root caries. Root-caries status was entered as the dependent outcome, while clinically relevant demographic, behavioral, periodontal, salivary, and biological-marker

variables were evaluated as independent variables. Variables considered included age, smoking status, diabetes mellitus, periodontitis, gingival recession, salivary pH, total antioxidant capacity, vitamin C, and MMP-8. Potential confounding variables were considered during model development. Results were reported as adjusted odds ratios (AORs) with 95% confidence intervals (CIs). Multicollinearity between independent variables was assessed before final interpretation of the regression model. A two-sided p-value <0.05 was considered statistically significant for all analyses.

RESULTS

A total of 430 participants/specimens were included in the analysis. The mean age was 48.7 ± 13.6 years, with the largest proportion belonging to the 40–59-year age group. Males accounted for 236 (54.9%) participants, while 194 (45.1%) were females. Root carious lesions were identified in 186 (43.3%) participants, whereas 244 (56.7%) had no clinically detectable root caries. Periodontitis was present in 258 (60.0%) participants. Coexistence of root caries and periodontitis was observed in 139 (32.3%) cases.

Table 1. Demographic and Clinical Characteristics of the Study Population (n = 430)

Variable	Frequency (%) / Mean $\pm$ SD
Age, years	48.7 $\pm$ 13.6
20–39 years	116 (27.0%)
40–59 years	201 (46.7%)
≥ 60 years	113 (26.3%)
Male	236 (54.9%)
Female	194 (45.1%)
Current smokers	104 (24.2%)
Diabetes mellitus	91 (21.2%)
Twice-daily tooth brushing	188 (43.7%)
Root caries present	186 (43.3%)
Periodontitis present	258 (60.0%)
Root caries with periodontitis	139 (32.3%)
Gingival recession present	232 (54.0%)

Among the 186 participants with root caries, single lesions were recorded in 82 (44.1%), while 104 (55.9%) had two or more lesions. Posterior teeth were more frequently affected than anterior teeth. Buccal root surfaces represented the commonest site of disease.

Active root lesions were observed in 111 (59.7%), whereas 75 (40.3%) lesions were classified as inactive. Moderate-to-deep root lesions were more frequent among participants with periodontal attachment loss.

Table 2. Characteristics of Root Carious Lesions (n = 186)

Characteristic	n (%)
Single root carious lesion	82 (44.1%)
Multiple root carious lesions	104 (55.9%)

Active lesions	111 (59.7%)
Inactive lesions	75 (40.3%)
Anterior teeth involved	49 (26.3%)
Posterior teeth involved	137 (73.7%)
Maxillary involvement	81 (43.5%)
Mandibular involvement	105 (56.5%)
Buccal surface	79 (42.5%)
Lingual/palatal surface	43 (23.1%)
Proximal surface	64 (34.4%)
Mild/shallow lesion	67 (36.0%)
Moderate lesion	76 (40.9%)
Deep lesion	43 (23.1%)

Periodontal parameters were significantly less favorable in individuals with root carious lesions. Participants with root carious lesion demonstrated a greater mean probing pocket depth, clinical attachment loss, gingival recession, plaque index and bleeding on probing compared with those without root

carious lesion. Mean probing pocket depth was 4.21 ± 1.16 mm in the root-caries group compared with 3.08 ± 0.91 mm in participants without root caries ($p < 0.001$). Similarly, mean clinical attachment loss was 4.69 ± 1.42 mm versus 2.91 ± 1.19 mm, respectively ($p < 0.001$).

Table 3. Periodontal Manifestation Traits According to Root Carious lesion Status

Periodontal variable	Root caries present (n=186)	Root caries absent (n=244)	p-value
Plaque Index	1.91 ± 0.55	1.37 ± 0.49	<0.001
Gingival Index	1.74 ± 0.52	1.22 ± 0.47	<0.001
Probing Pocket Depth, mm	4.21 ± 1.16	3.08 ± 0.91	<0.001
Clinical Attachment Loss, mm	4.69 ± 1.42	2.91 ± 1.19	<0.001
Gingival recession, mm	2.83 ± 1.25	1.41 ± 0.92	<0.001
Bleeding on probing, % sites	48.6 ± 19.7	30.9 ± 16.8	<0.001
Periodontitis, n (%)	139 (74.7%)	119 (48.8%)	<0.001

Assessment of oral biological markers demonstrated substantial differences between participants with and without root carious lesion. Individuals with root carious lesion had significantly lower salivary pH, salivary flow

rate, vitamin C concentration and total antioxidant capacity. In contrast, inflammatory biomarkers, including IL-1 β , IL-6, TNF- α and MMP-8, were higher among participants with root carious lesions.

Table 4. Oral Biological Marker Levels According to Root Carious lesion Status

Biomarker	Root caries present (n=186)	Root caries absent (n=244)	p-value
Salivary pH	6.31 ± 0.46	6.78 ± 0.43	<0.001
Salivary flow rate, mL/min	0.39 ± 0.16	0.56 ± 0.19	<0.001
Vitamin C, mg/dL	0.71 ± 0.25	0.93 ± 0.29	<0.001
Total antioxidant capacity, mmol/L	0.86 ± 0.27	1.13 ± 0.31	<0.001
IL-1 β , pg/mL	68.4 ± 22.7	49.6 ± 18.9	<0.001
IL-6, pg/mL	18.7 ± 7.8	12.8 ± 6.1	<0.001
TNF- α , pg/mL	15.9 ± 6.7	10.8 ± 5.2	<0.001
MMP-8, ng/mL	172.6 ± 57.4	116.3 ± 44.1	<0.001

When participants were classified according to periodontal status, inflammatory biomarkers increased progressively with worsening

periodontal disease. Participants with moderate-to-severe periodontitis had higher concentrations of IL-1 β , IL-6, TNF- α and MMP-

8, whereas vitamin C and total antioxidant capacity were comparatively reduced. The highest mean MMP-8 concentration was

observed among participants who had both root caries and periodontitis.

Table 5. Selected Biomarkers According to Combined Root Carious lesion and Periodontal Status

Biomarker	Neither condition (n=125)	Root caries only (n=47)	Periodontitis only (n=119)	Both conditions (n=139)	p-value
Salivary pH	6.89 ± 0.38	6.48 ± 0.41	6.66 ± 0.43	6.25 ± 0.44	<0.001
Vitamin C, mg/dL	0.99 ± 0.27	0.79 ± 0.25	0.86 ± 0.28	0.68 ± 0.24	<0.001
Total antioxidant capacity, mmol/L	1.21 ± 0.28	0.96 ± 0.25	1.04 ± 0.29	0.82 ± 0.25	<0.001
IL-1 β , pg/mL	43.7 ± 15.6	57.9 ± 18.7	55.8 ± 19.3	72.0 ± 22.9	<0.001
MMP-8, ng/mL	101.8 ± 36.7	138.6 ± 42.5	131.6 ± 43.9	184.1 ± 57.0	<0.001

Correlation analysis showed that root caries burden was positively related to clinical attachment loss ($r = 0.46$, $p < 0.001$), gingival recession ($r = 0.43$, $p < 0.001$), MMP-8 ($r = 0.39$, $p < 0.001$) and IL-1 β ($r = 0.34$, $p < 0.001$). In contrast, salivary pH ($r = -0.37$, $p < 0.001$), vitamin C ($r = -0.29$, $p < 0.001$) and total antioxidant capacity ($r = -0.32$, $p < 0.001$) showed inverse correlations with the number of root carious lesions.

A multivariable logistic regression model was constructed to identify factors independently associated with root caries. After adjustment for age, sex, smoking and diabetes, gingival recession, periodontitis, lower salivary pH and elevated MMP-8 remained independently associated with the presence of root carious lesion.

Table 6. Multivariable Logistic Regression for Factors Associated with Root Carious lesion

Variable	Adjusted OR	95% CI	p-value
Age ≥ 60 years	1.72	1.08–2.75	0.022
Current smoking	1.46	0.94–2.28	0.093
Diabetes mellitus	1.53	0.96–2.44	0.073
Periodontitis	2.18	1.42–3.35	<0.001
Gingival recession ≥ 2 mm	2.61	1.68–4.05	<0.001
Salivary pH < 6.5	2.27	1.49–3.46	<0.001
Low total antioxidant capacity	1.76	1.13–2.73	0.012
Elevated MMP-8	2.34	1.51–3.62	<0.001

Overall, the findings demonstrated a clear relationship between root carious lesions,

periodontal tissue destruction and alteration of the local oral biological environment.

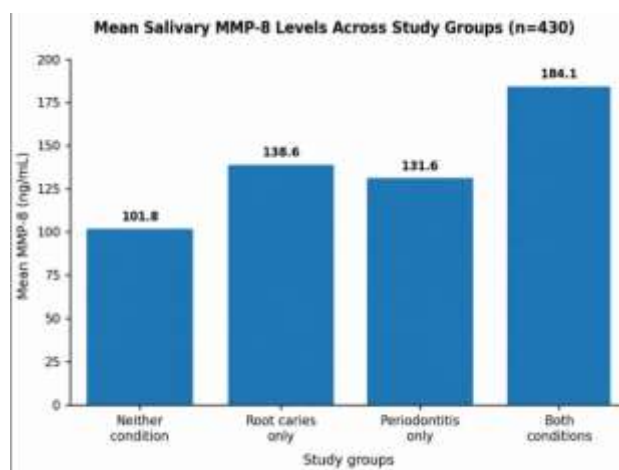


Figure 1. Mean salivary MMP-8 levels across study groups in the study population (n = 430).

The highest mean MMP-8 level was observed in participants with both root caries and periodontitis (184.1 ng/mL), followed by those with root carious lesion only (138.6 ng/mL) and periodontitis only (131.6 ng/mL), while

the lowest level was seen in participants with neither condition (101.8 ng/mL). The findings indicate greater inflammatory activity in subjects with coexisting root carious lesions and periodontal disease.

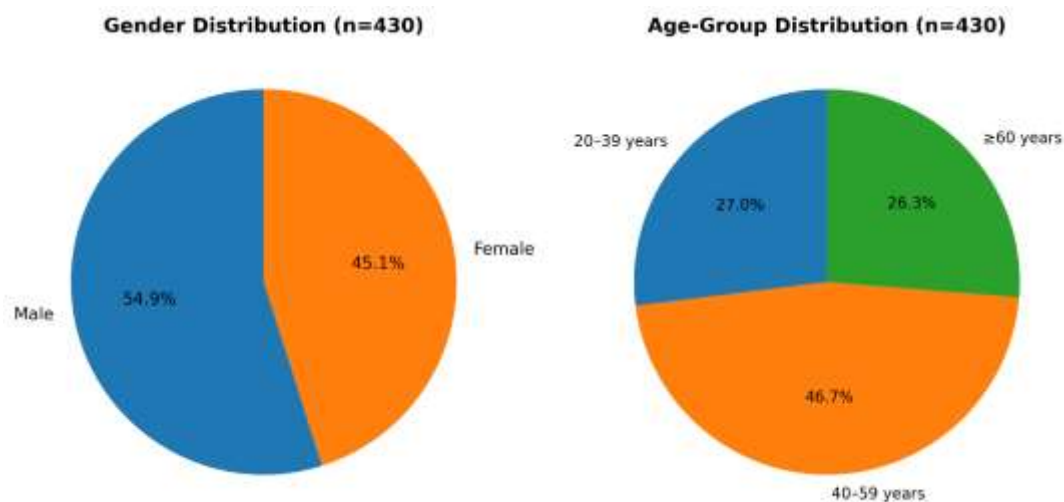


Figure 1. Distribution of study participants according to gender and age group (n = 430).

Males constituted 54.9% of the study population and females 45.1%. The largest age group was 40–59 years (46.7%), followed by 20–39 years (27.0%) and ≥60 years (26.3%). Assessment of Oral Biological M...

DISCUSSION

The present study demonstrated a considerable burden of both root carious lesion and periodontal disease, with root carious lesions identified in 43.3% of participants and periodontitis in 60.0%, while 32.3% showed coexistence of the two conditions. Participants with root carious lesion demonstrated greater plaque accumulation, gingival inflammation, probing pocket depth, clinical attachment loss, gingival recession, and bleeding on probing than participants without root carious lesion. These findings support the growing evidence that caries and periodontal disease, although biologically distinct conditions, may coexist because of shared behavioral, anatomical, and host-related determinants. Romandini et al. reported that adults with periodontitis had a greater occurrence and burden of untreated carious lesions, with subgroup analysis indicating a particularly relevant association with root carious lesion (18). Similarly, Li et al. found a positive clinical relationship between periodontitis and dental caries and reported a stronger association between periodontitis and root-surface caries than with coronal surface disease (19). Gingival recession and

periodontal attachment loss expose cementum and dentin to the oral environment, creating surfaces with greater vulnerability to cariogenic challenge. Root carious lesion continues to represent a substantial problem among adults retaining their natural teeth, particularly in aging populations (20,21).

More than half of participants with root carious lesion in the present study had multiple lesions, while posterior teeth and buccal surfaces showed greater involvement. Active lesions accounted for a substantial proportion of detected root caries. These findings highlight that exposed root surfaces can remain biologically active disease sites rather than representing only historical structural damage. Modern management of root carious lesion has consequently shifted toward early detection, preventive strategies, lesion arrest, and preservation of dental tissue. Evidence supports professionally applied fluoride therapies, including silver diamine fluoride and high-concentration fluoride preparations, for preventing or arresting root caries in susceptible older adults (22,23). Castelo et al. similarly emphasized conservative approaches for root-caries management and identified 38% silver diamine fluoride and high-fluoride products among interventions with clinically useful evidence (21). These findings reinforce the importance of identifying high-risk individuals before extensive cavitation occurs and suggest that biomarker-supported risk

assessment may eventually complement conventional clinical inspection.

A major observation of this study was the altered salivary environment among participants with root caries. Mean salivary pH and flow rate were lower among individuals with root carious lesions than among those without lesions. Saliva contributes to oral clearance, buffering, antimicrobial defense, mineral equilibrium, and protection against demineralization. Alteration of these properties may reduce the capacity of the oral environment to counter repeated cariogenic challenges. Contemporary systematic reviews have demonstrated that salivary molecular and physicochemical characteristics differ between individuals with and without dental caries, although considerable heterogeneity remains among available studies (24-27). Ahmad et al. reported differences in multiple salivary proteins between caries-active and caries-free individuals, while Alamoudi et al. concluded that salivary markers could have complementary value in caries detection and risk prediction (25,26). More recent evidence has further supported the potential of salivary biomarker profiles as non-invasive indicators of caries susceptibility, while emphasizing the need for standardized collection procedures and clinically validated thresholds (24,27).

The present research study findings also showed that participants with both root carious lesion and periodontitis had the most pronounced alteration in their biological-marker profile. Mean IL-1 β , IL-6, TNF- α , and MMP-8 concentrations were elevated, whereas antioxidant-related markers were reduced. This pattern suggests that coexistence of the two diseases may reflect a local environment characterized by enhanced inflammatory activity and impaired host protective mechanisms. A systematic review and meta-analysis of salivary profiles reported altered inflammatory-marker concentrations in individuals with dental caries, including differences in IL-6 and TNF- α (28). Saliva is increasingly recognized as a useful, minimally invasive diagnostic medium because it contains proteins, enzymes, cytokines, metabolites, immunologic factors, and microbial products capable of reflecting pathological processes within the oral cavity (29). However, available evidence does not yet support a single biomarker as sufficiently specific for routine diagnosis, suggesting that combined biological profiles may ultimately

have greater value than isolated measurements.

The association between inflammatory biomarkers and periodontal manifestations was particularly evident for MMP-8 and IL-1 β . Participants with both root caries and periodontitis had the highest mean MMP-8 concentration, while increasing MMP-8 and IL-1 β levels were positively correlated with greater disease burden. Protein-based salivary biomarkers have been extensively investigated in periodontology, and IL-1 β , MMP-8, IL-6, TNF- α , and related inflammatory mediators have demonstrated differences between periodontal health and disease (30). Domokos et al., in a systematic review and meta-analysis involving more than 1,700 individuals, found significantly higher salivary MMP-8 levels in periodontitis compared with periodontal health and reported relationships between MMP-8 and clinical periodontal parameters (31). The biological plausibility of this association is strong because MMP-8 participates in collagen breakdown and extracellular matrix degradation, which are central features of periodontal tissue destruction. Biomarker-based monitoring has therefore become an important area of precision dentistry, although methodological standardization remains necessary before widespread chairside implementation (32).

The antioxidant findings provide another relevant dimension to the observed relationship. Vitamin C and total antioxidant capacity were lower in the root-caries group and were particularly reduced among participants with simultaneous root caries and periodontitis. Oxidative stress develops when production of reactive oxygen species exceeds antioxidant defense, potentially amplifying inflammatory tissue injury. A meta-analysis by Mohideen et al. demonstrated greater oxidative stress in patients with periodontitis and generally reduced systemic or local antioxidant capacity compared with healthy controls (33). Vitamin C has antioxidant, collagen-supporting, and tissue-repair functions that may be particularly relevant to periodontal integrity. A recent systematic review examining ascorbic acid in oral and periodontal tissues reported potential improvements in periodontal parameters in several included clinical studies, although heterogeneity prevented definitive therapeutic conclusions (34). These data are compatible with the current observation that a less favorable antioxidant profile accompanied

more extensive periodontal and root-caries manifestations.

The relationship between salivary flow and oral disease should also be interpreted carefully. Lower salivary flow was identified in participants with root caries in the current study, which is biologically plausible because reduced clearance and buffering may prolong exposure of root dentin to cariogenic acids. However, reference values for normal salivary secretion vary considerably according to whether saliva is stimulated or unstimulated, collection technique, duration, time of day, age, medication use, and participant characteristics. A recent systematic review and meta-analysis specifically highlighted the methodological variability surrounding definitions of normal salivary flow in healthy adults (35). Accordingly, salivary flow rate may be most useful when interpreted alongside pH, disease activity, clinical examination, and other biological markers rather than as an isolated diagnostic measure.

The multivariable findings further demonstrated that periodontitis, gingival recession, lower salivary pH, and elevated MMP-8 remained independently associated with root carious lesion after adjustment for important covariates. Gingival recession appears particularly relevant because the root surface must first become exposed to the oral environment before conventional root carious lesion can develop. Periodontitis may therefore contribute indirectly through loss of periodontal attachment and root exposure while simultaneously contributing to an inflammatory oral environment. This interpretation is consistent with recent evidence syntheses specifically identifying an association between periodontitis and root carious lesion (18,19). The increasing global prevalence of dental caries among older adults and continued retention of natural teeth further emphasize the importance of integrated periodontal and caries risk assessment rather than treating the two conditions as completely independent clinical problems (36).

From a clinical perspective, the results suggest that patients presenting with periodontal attachment loss and gingival recession should undergo careful examination for early root-surface demineralization. Similarly, patients with multiple or active root carious lesions may benefit from comprehensive periodontal assessment. Biological-marker evaluation may eventually provide an additional level of risk

stratification, particularly where conventional clinical findings alone do not fully reflect ongoing disease activity. Nevertheless, current evidence remains insufficient to recommend salivary biomarker panels as substitutes for established clinical diagnostic criteria. Reviews of salivary diagnostics consistently emphasize their potential while also identifying heterogeneity in sampling, laboratory methods, diagnostic thresholds, and study populations as major barriers to routine application (24,27,29,32). Future approaches should therefore combine clinical findings with standardized biological measurements, rather than interpreting biomarkers in isolation.

The present research study also contributes to the literature by evaluating root carious lesions, periodontal manifestations, salivary physicochemical characteristics, inflammatory mediators, and antioxidant markers within the same relatively large study population. This multimarker approach provides a more integrated view of the biological environment associated with coexisting root caries and periodontal disease. However, the cross-sectional design prevents determination of whether the biomarker alterations preceded disease development or occurred as a consequence of established lesions. Large prospective cohorts should evaluate whether combinations of MMP-8, IL-1 β , antioxidant measures, salivary pH, and salivary flow can prospectively identify individuals at increased risk of developing new root lesions or periodontal deterioration. External validation across different populations is also necessary before diagnostic cut-offs can be proposed. The long-term success of root-caries management remains influenced by lesion behavior, preventive care, and restoration longevity, further supporting the need for longitudinal follow-up rather than reliance on single-point assessment (37).

Limitations of the Study

The findings of the present research study should be interpreted in view of certain limitations. The Cross-sectional analytical design allowed identification of associations between oral biological markers, root caries, and periodontal manifestations but did not establish a temporal or causal relationship. Participants were recruited from a single dental center, which may restrict generalizability to populations with different demographic, dietary, socioeconomic, and oral-health characteristics. Salivary biomarkers

may also be influenced by factors such as hydration, diet, circadian variation, smoking, systemic disease, medications, and recent oral-hygiene practices despite standardized sample-collection procedures. Furthermore, although several clinically relevant inflammatory and antioxidant biomarkers were assessed, the study did not incorporate comprehensive microbiome, metabolomic, or proteomic profiling. Longitudinal multicenter investigations are therefore required to determine whether the identified biomarker combinations can predict the development or progression of root carious lesion and periodontitis.

CONCLUSION

The present research study demonstrated a significant relationship between root carious lesion, periodontal tissue destruction, and alterations in the oral biological environment. Participants having root carious lesions showed greater probing pocket depth, clinical attachment loss, gingival recession, plaque accumulation, and bleeding on probing, while individuals with simultaneous root carious lesion and periodontitis demonstrated the most pronounced inflammatory biomarker profile. Increased MMP-8, IL-1 β , IL-6, and TNF- α , together with reduced vitamin C, total antioxidant capacity, salivary pH, and salivary flow, characterized a less favorable oral biological environment. Periodontitis, gingival recession, reduced salivary pH, and elevated MMP-8 remained important factors associated with root carious lesion in multivariable analysis. These findings support an integrated clinical approach in which root carious lesion assessment and periodontal evaluation are performed together. Salivary biomarkers may provide useful supplementary information for future risk assessment; however, prospective multicenter studies and standardized biomarker thresholds are needed before they can be incorporated into routine diagnostic practice.

Informed Consent: As per International standard and University standard, Participants written Informed consent has been collected and preserved by the authors (s) included in this research study.

Ethical Approval: As per International standard and University standard; a written Ethical approval was obtained from the relevant Institutional Research and Ethical Review Committee of Shahida Islam Medical Complex, Lodhran.

Conflict of Interest: The authors declare that they have no conflict of interest.

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